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February 3, 2006

Dr. Michael D. Shelby, CERHR Director
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Submitted electronically to shelby@niehs.nih.gov

Dear Dr. Shelby,

The following comments are submitted on behalf of the more than one million members and supporters of People for the Ethical Treatment of Animals (PETA), in response to the NTP-CERHR expert panel update on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate (DEHP) which was prepared in November, 2005. PETA is the world's largest animal rights organization and is committed to using the best available science to protect animals from suffering and to promote the acceptance of alternatives to animal testing.

Summary of comments

Recommendations for several of the data needs listed in the update's summaries and conclusions section call for additional studies on animals, including primates. However, as noted in the American Chemistry Council (ACC) comments to the draft update (2005), these data needs have been sufficiently met by existing studies, including a number of recent studies that do not appear to have been fully considered by the expert panel. Existing data clearly demonstrate that primates, including humans, are much less sensitive than rats to the developmental and reproductive effects of DEHP. Considering that estimates of human exposures calculated by the ACC (2005) from recent CDC biomonitoring data are 1,000 to 10,000-fold lower than NOELs determined in rats, the existing weight of evidence is clearly sufficient to establish conservative NOELs for all relevant human populations and exposures to safeguard the public health without subjecting additional animals to suffering and death.

Low level dose-response data exist for DEHP and MEHP

Under the heading "Significance of Perinatal Exposure", section 3.1, the need for additional dose-response data correlating mono-(2-ethylhexyl) phthalate (MEHP) levels and developmental reproductive effects in rats is identified. However, the dose-responses of rats to DEHP and its toxicologically active metabolite MEHP at low levels are well-characterized and sufficient to establish a developmental NOEL of 46 mg/kg. Li et al. (2000) investigated the effects of low doses of DEHP and MEHP on the testicular development of rat pups. DEHP doses of 20, 100, 300 and 500 mg/kg were administered to 3-day-old rat pups as a single dose by oral gavage. The investigators found that the lowest dose of DEHP that produced changes in neonatal testicular cells was 100 mg/kg.

The results of a recent NTP continuous breeding study (2005) are consistent with these findings. Although the purpose of this study was to assess potential reproductive effects, developmental effects were also measured. DEHP doses of 10, 30, 100, 300, 1,000, 7,500, and 10,000 ppm were administered in feed to groups of 17 male and 17 female rats. Reproductive effects were noted in the 7500 ppm and 10,000 ppm groups. Developmental effects, such as decreases in the weights of sex organs and histological abnormalities, were also observed at these concentrations. While mention is made of small sex organs in several rat pups in the 300 and 1000 ppm groups, the authors viewed the toxicological significance of these findings as questionable since organ weights were normal, no other reproductive effects were noted, and the incidence of these findings was low. As a result, the developmental NOEL, as suggested by the expert panel, is most likely 1000 ppm, calculated to be no more than 46 mg/kg/day based on feed consumption.

The effect of intravenous exposure to DEHP on the development of reproductive organs in male rat pups was investigated by Cammack et al. (2003). No effects of any kind were observed in animals treated with 60 mg/kg/day, while testicular changes were noted in the 300 and 600 mg/kg/day dose groups. Other groups were dosed daily by oral gavage at 300 and 600 mg/kg/day. The investigators noted that testes changes were generally more severe among animals dosed orally than intravenously. These consistent findings across studies support a conservative developmental reproductive NOEL of 46 mg/kg/day for oral exposure to DEHP and 60 mg/kg/day for intravenous exposure.

ADME data in primates explains reduced sensitivity

Under the heading "Extension of PBPK Model", section 4, the need to extend ADME data across species into primates is identified and under the "Additional Data Needs" heading *in vivo* metabolic data on lipase across species is listed. There is ample data to conclude that primates are much less sensitive to the developmental and reproductive effects of DEHP than are rodents. In addition, the mechanisms responsible for this lower sensitivity can be understood on the basis of existing ADME data. A recent study by Tomonari et al. (2004) found that exposure to very high levels of DEHP – 2500 mg/kg/day – resulted in no observed effects on testicular development in marmosets. In addition, a recent human study found no developmental effects of DEHP in adolescents who had been exposed to high medical treatment related levels as neonates (Rais-Bahrami et al., 2004).

Studies by Rhodes et al. (1986), Astill (1989) and Kurata et al. (2005) demonstrated that DEHP is absorbed 10 to 100-fold less efficiently in marmosets and cynomolgous monkeys than in rats. Furthermore, at higher doses absorption efficiency decreased with the peak blood level for MEHP in marmosets leveling off at 20 mg/L. Lipase is the enzyme that catalyzes the hydrolysis of DEHP to its toxicologically active metabolite MEHP. Ito et al. (2005) found that lipase activity in rats was more than 10-fold higher than in marmosets, and the Vmax/Km ratio was nearly 200-fold greater. In addition, MEHP and its metabolites remained in their more active, free forms in rodents, while in primates they were conjugated with glucuronide (Silva et al., 2003; Kato et al., 2004; Kurata et al., 2005). Glucuronide conjugation decreases their toxicological activity and increases their water solubility resulting in faster excretion in the urine. This conclusion is supported by the results of Kessler et al. (2004) who demonstrated that while the area under the plasma concentration versus time curves (AUCs) for MEHP was 3 to 10-fold higher in rats than in marmosets, the peak blood level was only 1 to 3-fold higher, a result of faster excretion of MEHP in marmosets. The observed lower sensitivity of primates to the developmental and reproductive effects of DEHP can therefore be explained by less efficient absorption, lower activity of lipase, and increased glucuronidation of MEHP and its metabolites resulting in faster excretion. This explanation is consistent with the observation that the AUC for MEHP was 100-fold higher than the AUC for DEHP in rats but only 10-fold higher in marmosets (Kessler, et al., 2004). Also, a much higher proportion of oral DEHP doses was excreted as DEHP in the feces in marmosets than in rats.

Estimated human exposures are 1,000 to 10,000-fold lower than the experimental NOEL

In its comments to the draft update, the ACC calculated human exposure to DEHP based on recent CDC urinary metabolite biomonitoring data. The range of exposures for all U.S. populations was conservatively estimated to be 3-30 μg/kg/day. This range is 1,000 to 10,000-fold lower than the experimentally determined rat oral NOEL of 46mg/kg/day.

The need for additional dose-response data correlating MEHP levels and developmental reproductive effects in rats has been met by studies of Li et al. (2000), Cammack et al. (2003) and NTP (2005). The results of each of these studies are consistent with establishing a conservative developmental reproductive NOEL in rats of 46 mg/kg/day for oral exposure to DEHP and 60 mg/kg/day for intravenous exposure. Developmental reproductive effects of DEHP exposure were not observed in humans (Rais-Bahrami et al., 2004) or other primates (Tomonari et al., 2004) even at much higher exposure levels, such as those that have been found in medical treatment-related exposures.

This lower sensitivity is adequately explained by species differences in the absorption, metabolism and excretion of DEHP and its active metabolites (Rhodes et al., 1986; Astill, 1989; Silva et al., 2003; Kato et al., 2004; Kurata et al., 2005). Considering that estimates of human exposures calculated from recent CDC biomonitoring data are 1,000 to 10,000-fold lower than NOELs determined in rats, the NTP can reasonably conclude that more animal data will not increase public safety and that the developmental NOELs based on existing data are sufficient.

Please feel free to contact me at 610-586-3975 or via e-mail at JosephM@peta.org if you have any questions.

Sincerely,

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